

THE ACTION OF EYE-STALK EXTRACTS ON RETINAL PIGMENT MIGRATION IN THE CRAYFISH, *CAMBARUS BARTONI*

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I

Pigment cells of the retina of the vertebrate eye and pigment cells of the compound eye of arthropods have long been known to lack motor innervation. Hence there has been much speculation regarding the nature of the mechanisms controlling the movements of these cells or the pigment within them. As recently as 1932 when Parker reviewed the literature on retinal pigments there was no direct evidence as to the nature of the control, but considerable indirect evidence suggested that hormonal agents were responsible for initiating and maintaining retinal pigment migration. The first successful attempt to demonstrate the existence of a hormone acting on the retinal pigments of arthropods was made by Kleinholz (1934; 1936). He found that the injection of an active principle from eye-stalks of *Palae-moneles* into dark-adapted individuals of the same species caused the movement of the distal and reflecting pigments to positions characteristic of the light.

Studies of the persistence, under constant external conditions, of 24-hour cycles of pigment migration in the compound eye had led to one of the earlier suggestions that there was a hormonal control of retinal pigment (Welsh, 1930; see also Welsh, 1938, for review of the literature pertaining to diurnal rhythms). The extension of these studies to the eye of *Cambarus* made necessary an investigation of hormone factors in the control of retinal pigment migration in this crustacean. Certain of the results obtained will be presented in this paper.

II

The majority of observations were made on eyes of *Cambarus bartoni* but eye-stalks of *C. clarkii* and *C. limosus* were sometimes used as sources of the pigment-activating substance.

The approximate positions of the three sets of pigment (distal, proximal and reflecting) were determined by briefly illuminating the

eye of an animal, in the dark, by a bright beam of light, and observing the amount of light reflected from the eye. This method has been employed by Day (1911). Exact determinations of pigment positions were first made by sectioning the eyes but this is a time-consuming procedure and a rapid method was developed as follows. Animals were killed by dipping in water at 80° C. for 10–15 seconds. The eyes were then removed and split in halves. When these halves were examined under a binocular, using bright reflected light, it was possible to measure the positions of the pigments quite as accurately as in sections.

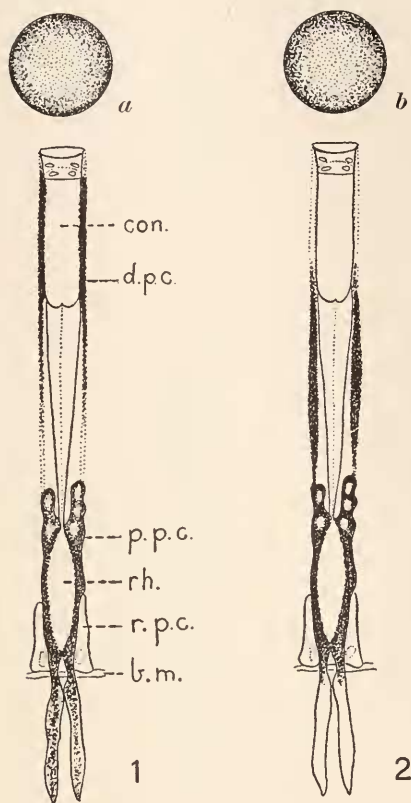
The active substance from the eye-stalk, which may be identical with the chromatophorotropic hormone (Abramowitz and Abramowitz, 1938), was prepared by grinding 20 eyes and eye-stalks of medium-sized crayfishes in 1 cc. of cold-blooded Ringer, then heating to 100° C. and filtering. The injection of an appropriate volume of the filtrate made it possible to administer the active material from a fraction of an eye-stalk or from one or more eye-stalks as a given dose. Appropriate control injections of Ringer's fluid and of extracts of ventral nerve cord were made and always with negative results.

III

The first experiments to be reported were done to test the effect of the eye-stalk extract on light-adapted eyes. Several *C. bartoni* were allowed to adapt for several hours in bright diffuse sunlight. At 10:30 A.M. 0.05 cc. of eye-stalk extract (containing the active material from one eye-stalk) was injected in the ventral abdominal musculature of each of half the individuals. At 2:00 P.M. the entire lot was killed with hot water and the eyes removed, split and examined.

In Fig. 1 may be seen the distribution of the pigments of a light-adapted eye. The distal pigment forms a sheath around the cone and the process leading from the cone to the rhabdome, but a portion of each distal pigment cell next to the reticular or proximal pigment cells is not filled with pigment. Most of the proximal pigment surrounds the rhabdome, but some remains below the basement membrane. The reflecting pigment in crayfish eyes does not migrate as it does in some crustaceans (Welsh, 1932). The appearance of an eye of a light-adapted animal, when viewed by reflected light, is shown in Fig. 1a. The positions of the black screening pigments are such that light cannot reach the reflecting pigment layer nor can light rays, except those which are parallel to the main axis of an ommatidium, reach the rhabdome or light-sensitive element of the eye. Such an eye is called an apposition eye since a given rhabdome receives light

only from its adjacent lens system and is not acted on by light entering at an angle through neighboring ommatidia.



EXPLANATION OF FIGURES

- con.* = cone
b.m. = basement membrane
d.p.c. = distal pigment cell
p.p.c. = proximal pigment cell
rh. = rhabdome
r.p.c. = reflecting pigment cell

FIG. 1. An ommatidium of a typical light-adapted eye showing the positions of the eye pigments. This and the following figures of ommatidia show the situation as seen in thin sections of the eye. In the intact light-adapted eye each cone, cone process and rhabdome is almost completely surrounded by a cylinder of pigment.

FIG. 1a. Showing the appearance of an intact eye with the pigment distribution seen in Fig. 1 when viewed, in the dark, by bright reflected light.

FIG. 2. An ommatidium showing the effect on the pigments of injection of eye-stalk extract into a light-adapted animal.

FIG. 2b. The intact eye has essentially the same appearance as does the normal light-adapted eye.

An ommatidium from a typical eye of a light-adapted animal, injected with eye-stalk extract and left in the light, is represented by Fig. 2. The distal pigment is in a more extreme proximal position and all of the proximal pigment is above the basement membrane. It is as though the effect of the injected material were added to the effect of light, which probably acts by causing the release of the active material or hormone. The intact eye of such an animal has essentially the same appearance as does the normal light-adapted eye (Fig. 2*b*), although it may not be as black. This is due to the distance of the distal pigment from the surface of the eye.

IV

When specimens of *C. bartoni* were placed in the dark, the typical dark-adapted condition in the eye was seen after two hours or less. It is known, however, that there is a diurnal migration of proximal retinal pigment in crayfishes which are kept in continuous darkness (Bennitt, 1932), so in order to assure uniform conditions in all experiments on dark-adapted animals the majority of observations were made in the early evening.

The positions normally occupied by pigments in a dark-adapted eye are shown in Fig. 3. The distal pigment forms a collar surrounding the cone and the proximal pigment is all below the basement membrane. In such a condition the rhabdome of a given ommatidium may receive light from neighboring ommatidia. Such an eye is referred to as a superposition eye and is commonly found in those insects and crustaceans which are active at night.

The intact eye of a dark-adapted crayfish has a brilliant orange-red center when viewed by reflected light, due to the mirror-like property of the exposed reflecting or tapetal layer (Fig. 3*c*). The color is due to the visual red of the rhabdomes.

When dark-adapted *C. bartoni* were injected with eye-stalk extracts, and left in the dark, varying effects on the pigment were seen depending on the amount injected, and the interval between the time of injection and the time of observation. Animals which were dark-adapted for several hours and injected in the early evening with an amount of material equivalent to that obtained from one-fourth to one eye-stalk showed, after three hours, a migration of the distal pigment to or toward the light position. The proximal pigment was not affected (Fig. 4). When such eyes are viewed by reflected light they appear gray rather than black and very little light is reflected from the tapetal layer (Fig. 4*d*).

The injection of 0.1 cc. of the extract (= the extractible material from two eye-stalks) had a distinct effect on the proximal as well as

the distal pigment. After three hours both pigments were found to occupy positions more or less typical of light adaptation (Fig. 5). The intact eye when viewed by reflected light had the same appearance as the normal light-adapted eye (cf. Fig. 5*e* with 1*a*).

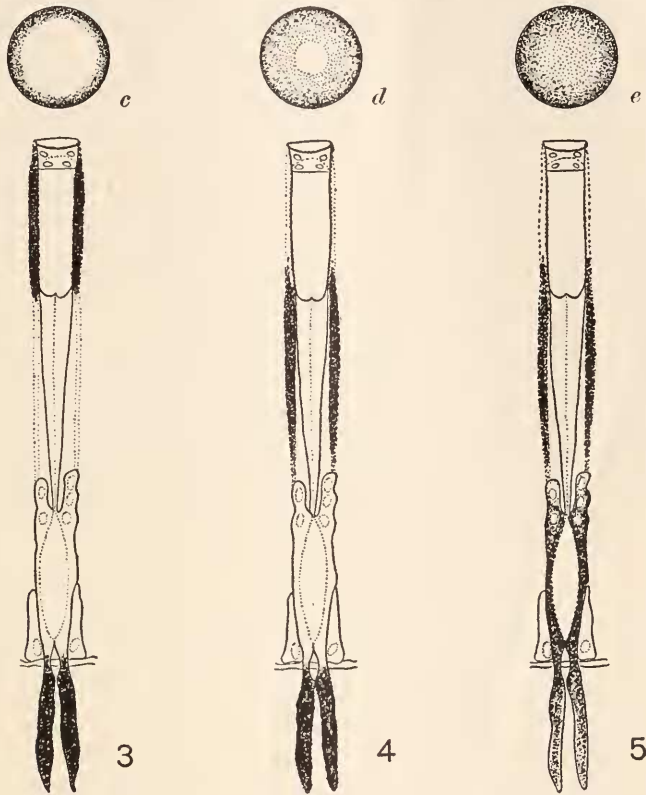


FIG. 3. Ommatidium of a typical dark-adapted eye showing the positions of the pigments.

FIG. 3*c*. The intact eye of a dark-adapted animal has a bright orange-red center when viewed by reflected light.

FIG. 4. The injection of the active material from one eye-stalk into a dark-adapted animal causes the migration of the distal pigment to the light position.

FIG. 4*d*. The intact eye of such an animal may have a small reflecting central area.

FIG. 5. The injection of the active material from two eye-stalks into a dark-adapted animal causes the migration of both distal and proximal pigments to their light positions.

FIG. 5*e*. The intact eye of such an animal has the same appearance as that of a light-adapted animal.

V

It has been demonstrated that it is possible, by means of a simple extraction process, to obtain from two eye-stalks of a crayfish an amount of retinal pigment activator, or hormone, equivalent to that normally released by the animal during the process of light adaptation. From one eye-stalk the amount of hormone is sufficient only to activate the distal pigment cells; thus indicating that they have a lower threshold than do the proximal pigment cells. Such threshold differences between the three sets of pigments in a given species may account for such a situation as was first seen in the eye of *Macrobrachium* (Welsh, 1930), where under continuous illumination the distal pigment cells migrate toward the periphery of the eye at the time of sunset and return to a proximal position at the time of sunrise, while the proximal pigment remains in a constant light position (see also Welsh, 1935, 1936; and Kleinholz, 1937, 1938).

The injection of eye-stalk extracts into dark-adapted crayfishes makes it possible to obtain a "light-adapted" eye, as regards the positions of the screening pigments, while the "dark-adapted" level of the light-sensitive substance of the retina remains unaffected. This enables one to study the effect of pigment position on visual acuity and response to flicker and has been employed by Crozier and Wolf (1939).

SUMMARY

A substance similar to, or identical with, the eye-stalk or chromatophorotropic hormone may be obtained from the eye-stalks of crayfishes. When injected, in proper amount, into light-adapted crayfishes it causes the distal and proximal pigments to migrate to more extreme "light positions" than normal. When injected into dark-adapted crayfishes which are allowed to remain in the dark it causes the migration of one or both sets of screening pigment to their "light positions." The distal pigment has a lower threshold than the proximal pigment, as it is affected by lower concentrations of the active substance. It is suggested that such threshold differences may account, in part, for the unusual pigment responses which have been observed in compound eyes in studies of 24-hour cycles in pigment migration.

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